Effect of Motility of Phytophthora palmivora Zoospores on Disease Severity in Papaya Seedlings and Substrate Colonization in Soil

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ABSTRACT

Zoospores of *Phytophthora palmivora* exhibited a tactic response to roots, but not to autoclaved stems, of papaya seedlings on natural soil. Motile zoospores were more effective in killing seedlings than nonmotile zoospores in soil, indicating that zoospore attraction towards plant roots is of importance in disease development. However, due to the difference in speed of germination, nonmotile zoospores were more effective in colonizing autoclaved papaya stems than motile zoospores.

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Additional key words: Carica papaya, microsyringe method, vertical-illumination microscope.

Although attraction of zoospores towards plant roots in water is common in plant pathogenic Phycomycetes, little is known about zoospore behavior in nature (3). Ho (4) reported the accumulation of zoospores of *Phytophthora megasperma* var. *sojae* on roots grown in quartz sand. A nonspecific accumulation of zoospores of *Phytophthora drechsleri* and *P. megasperma* var. *sojae* on roots of both host and nonhost plants in soil also has been demonstrated (9). However, the significance of such accumulation in disease development has not been determined. In this paper the effect of zoospore motility on severity of disease was explored. Since little attention has been paid to the attraction of zoospores toward dead organic matter, we also investigated the effect of zoospore motility on the degree of substrate colonization.

MATERIALS AND METHODS.—*Observation of zoospore attraction.—Phytophthora palmivora* Butler (isolate 18F-2P) was obtained from an infected root of papaya (*Carica papaya* L.). Production of sporangia was induced by growing the fungus on V-8 juice agar under fluorescent light for 7 days at 24 C (1). A sporangial suspension was obtained by spraying culture plates with distilled water using an atomizer. After 1 h of incubation at 16 C, released zoospores were separated from sporangia by passing the suspension through a 20-μm screen (Buckbee-Mears Co.). The zoospore concn was determined by the microsyringe method (6). Two drops (1 μl each) of the diluted spore suspension were placed on a glass slide and zoospores were counted under a microscope with a ×10 objective.

To observe zoospore attraction, a 1-mo-old papaya seedling was carefully removed from soil and adhering soil particles were removed by immersing roots in tap water. The seedling was placed in a small petri dish (60 × 15 mm) containing 5 ml zoospore suspension, with the stem leaning on the edge and roots resting on the bottom of the dish. Attraction of zoospores to roots was observed with a light microscope.

The vertical-illumination microscope technique (5) was used to observe zoospore attraction to roots directly on soil. A sandy loam soil (pH 5.9) collected from papaya production area was used throughout the experiments. Approximately 4 g of soil was placed on a glass slide, compressed, and the surface smoothed with a spatula to give a final soil volume of about 35 × 20 × 4 mm. Two slides with soil were placed in a large petri dish (150 × 20 mm), and approximately 0.03 ml of zoospore suspension was pipetted onto each soil surface. A papaya seedling was then placed between the two slides with roots resting on the soil surfaces. Attraction of zoospores towards roots on soil was observed with a Zeiss Universal Microscope equipped with a Model II C vertical illuminator and ×16 Epiplan objective. A heat reflection filter was used to reduce the heat generated. For determining duration of zoospore motility in soil, 0.2 ml of zoospore suspension (5.5×10^5/ml) was pipetted onto 2 g of soil, resulting in a soil moisture content of approximately 70% water-holding capacity. At 30-min intervals, 0.2 g of soil was suspended in 1 ml of water in a small petri dish and observed microscopically.

Effect of zoospore motility on disease severity.—Twenty-five papaya seeds were planted in soil in a 2-liter plastic container. After 4 wk, seedlings were inoculated by evenly distributing 50 ml of zoospore suspension over the soil surface. Three 10-fold dilutions and three replicates for each dilution were used for each treatment. Seedlings were watered and wilted seedlings removed twice daily. The experiments were repeated twice.

Effect of zoospore motility on substrate colonization.—Fifty ml of zoospore suspension was thoroughly mixed with 200 ml of soil in a 400-ml beaker. The final moisture content of the soil was about 45% water-holding capacity. Fifty papaya stem sections (10 mm long) from 2- to 3-mo-old seedlings were distributed in the inoculated soil. Beakers were covered with petri dish lids. After 24 h of incubation at 24 C, stem sections were removed from soil and washed in running tap water for 30 min. Stem sections were then placed on a selective medium (8). The presence of *P. palmivora* in stem sections was indicated by the production of sporangia after incubation at 24 C under light for 3 days. To study the effect of pre-incubation of the substrate in soil on colonization by zoospores, autoclaved stem sections were incubated in noninoculated soil for 4.5 h, removed from soil, and used as previously described. The experiments were done at least twice.

RESULTS.—*Effect of zoospore motility on disease severity.*—Attraction of zoospores of *P. palmivora* towards papaya roots in water occurred within 1.0 min.

Root surfaces were subsequently covered by masses of encysted zoospores (Fig. 1-A). Attraction of zoospores towards roots and their accumulation on root surfaces also occurred on soil (Fig. 1-C). When longevity of zoospore motility in soil was tested, only a few slowly-moving zoospores were observed after 4 h; no motility was observed at 4.5 h. Therefore, *P. palmivora* zoospores and papaya seedlings were considered suitable materials for studying the effect of zoospore motility in soil on disease severity.

To obtain nonmotile zoospore for comparison, zoospore motility was terminated by agitating the spore suspension in a test tube for 1-1.5 min with a Vortex mixer (10). No bursting of zoospores occurred and both motile and nonmotile zoospores germinated completely on V-8 juice agar with no difference in size and shape of germ tubes. Motile zoospores were more effective in inciting disease than nonmotile zoospores (Fig. 2). The percentage of seedlings killed by motile and nonmotile zoospores was 94 and 22, respectively, at 9×10^5 spores/container. At a concn of 9×10^4 spores/container, the difference between percentage of seedlings killed by motile and nonmotile zoospores was 56%, which was very close to the difference (57%) between percentage of seedlings killed by motile zoospores at 9×10^4 and 9×10^3 spore/container. Therefore, under conditions tested, the effect of zoospore immobilization was similar to that of decreasing the zoospore population 10-fold. Zoospore encystment was also induced by incubating zoospore suspensions at 33 C for 0.5 h or at 28 C for 2.5 h. The temp treatments did not affect subsequent germination. Again, motile zoospores were more effective in killing seedlings than encysted zoospores (Table 1). For instance, at a concn of 6×10^4/container, 98% of the seedlings were killed when motile zoospores were used, but only 11% and 9% of the seedlings were killed when encysted zoospores, induced by temps of 33 C and 28 C, respectively, were used.

Effect of zoospore motility on substrate colonization.—For studying the degree of substrate colonization of *P. palmivora* zoospores, spore motility was terminated by agitation as described previously. In contrast to results of disease severity studies, nonmotile zoospores were more effective in colonizing autoclaved papaya stems than motile zoospores. Percentages of stems colonized by motile and nonmotile zoospores were 0 and 88, respectively, at a concn of 9×10^4/container. At the concn of 9×10^4 spores/container, the difference between percentage of stems colonized by motile and nonmotile zoospores was 88%, which was the same as the difference between percentage of stems colonized by nonmotile zoospores at 9×10^4 and 9×10^3 spores/container. Therefore, under such conditions the effect of terminating zoospore motility on colonization of autoclaved papaya stems, was similar to that of increasing the zoospore population 10-fold. Autoclaved papaya stems did not attract zoospores either in water (Fig. 1-B) or directly on soil. Contrary to autoclaved stems, living papaya stems were attractive to *P. palmivora* zoospores and colonization of living stems by motile and nonmotile zoospores was similar. At the concn of 1.2×10^5 spores/container, the percentage of stems colonized by motile and nonmotile zoospores were 52 and 48, respectively.

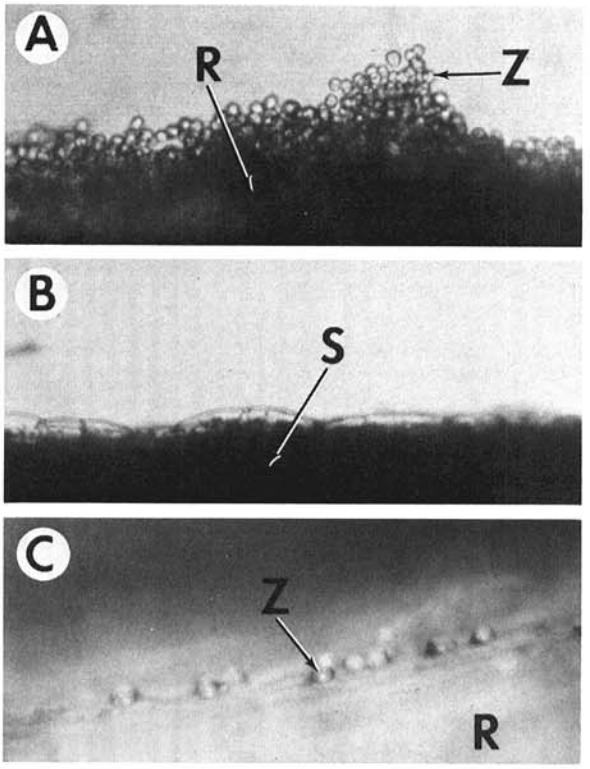

Fig. 1-(A to C). A) Accumulation of zoospores (Z) of *Phytophthora palmivora* on a root (R) of papaya seedling in water (×327). **B)** Nonaccumulation of zoospores on an autoclaved papaya stem (S) in water (×327). **C)** Accumulation of zoospores (Z) on a papaya root (R) on soil (S) (×414).

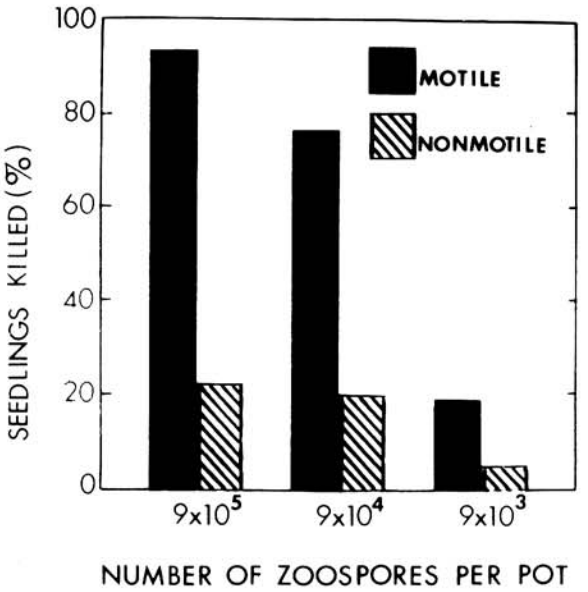

Fig. 2. Effect of motility of *Phytophthora palmivora* zoospores on disease severity in papaya seedlings. Zoospore motility was terminated by agitation for 1-1.5 min with a Vortex mixer.

TABLE 1. Effect of zoospore motility of *Phytophthora palmivora* on disease severity in papaya seedlings

Zoospore concn (No./container)	Seedlings killed (%)[a]		
	Motile	Nonmotile (33 C)[b]	Nonmotile (28 C)[b]
6×10^5	100	53	28
6×10^4	98	11	9
6×10^3	78	--	9

[a]Based on three replications with 25 seedlings/replication.
[b]Zoospore motility was terminated by incubation at 33 C for 0.5 h or 28 C for 2.5 h.

Preincubation of stems in natural soil also decreased stem colonization by zoospores. At the concn of 5.5×10^5 spores/container the percentage of stems colonized by nonmotile zoospores in natural soil decreased from 92 with nontreated stems to 62 with preincubated stems.

DISCUSSION.—Zoospores of *P. palmivora* remained motile for 4.5 h and exhibited tactic response to papaya roots on natural soil. The significance of attraction of zoospores towards plant roots in disease development was demonstrated by comparing the disease severity induced by motile and nonmotile zoospores of *P. palmivora* on papaya seedlings. Zoospores induced to encyst by agitation with a Vortex mixer, or by incubation at 28 or 33 C, were less infective than motile zoospores. Zoospores apparently were not damaged by agitation because agitation causes withdrawing rather than shedding of flagella (10). Moreover, the agitated zoospores germinated completely on nutrient agar and were more effective in colonizing papaya stems than the nonagitated zoospores.

Zoospores of *P. palmivora* did not exhibit tactic response to autoclaved papaya stems and their ability to colonize stems was not decreased by terminating their motility. The nonmotile zoospores colonized substrates more readily than motile zoospores. This may be attributed to the difference in speed of germination between nonmotile and motile zoospores (2). Zoospore germination occurs only after encystment. Consequently, nonmotile zoospores would germinate faster than motile ones in soil. There was no significant difference between colonization of living tissues by motile and nonmotile zoospores. Apparently, the advantage of mobility and tactic response to living tissues, compensated for the disadvantage of slow germination of motile zoospores in substrate colonization.

Ability of *P. palmivora* zoospores to colonize papaya stems was decreased by preincubation of stems with natural soil. Such reduction with preincubation may be due to competition of soil microorganisms for soluble nutrients released from the stems. This possibility is supported by a previous study which showed that agar disks incubated on soil lost 30-50% of carbohydrates and amino acids within 2 h (7).

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